Thermal destruction of *Escherichia coli* O157:H7 in beef and chicken: determination of D- and z-values[1]

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Abstract

Thermal inactivation of a four-strain mixture of *E. coli* 0157:H7 was determined in 90% lean ground beef, and lean ground chicken. Inoculated meat was packaged in bags which were completely immersed in a circulating water bath and held at 55, 57.5, 60, 62.5, and 65°C for predetermined lengths of time. D-values, determined by linear regression, in beef were 21.13, 4.95, 3.17, 0.93 and .0.39 min, respectively (z = 6.0°C). Using a survival model for non-linear survival curves, D-values in beef ranged from 20.45 min (D_1; and there was no D_2) at 55°C to 0.16 min (D_1) and 1.45 min (D_2) at 65°C. When *E. coli* O157:H7 four-strain cocktail was heated in chicken, D-values calculated by both approaches were consistently less at all temperatures. The heat resistance of *E. coli* O157:H7 was not altered after refrigerated or frozen storage of inoculated beef for 48 h. The results of this study will be beneficial to the food industry in designing HACCP plans to effectively eliminate *E. coli* 0157:H7 in the meat products used in this study.

1. Introduction

Escherichia coli O157:H7, a facultative anaerobe, is an etiological agent of hemorrhagic colitis, and the life-threatening post-diarrhoeal complications of hemolytic uremic syndrome (Tarr, 1994). Recent surveys of cattle herds and meat plants have clearly shown that the organism is not present routinely in carcass and raw ground beef

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samples (USDA-FSIS, 1996a,b). For example when the prevalence of *E. coli* O157:H7 was examined in cow and beef carcasses from December 1993 through November 1994, the organism was not recovered from any of the 2112 carcasses (USDA-FSIS, 1996a). Also, *E. coli* O157:H7 was not recovered from any of the 563 raw ground beef samples collected from meat plants (USDA-FSIS, 1996b). Nonetheless, while these reports clearly indicate that the organism is a rare contaminant in beef, 25 out of 63 foodborne disease outbreaks (40%) associated with *E. coli* O157:H7 from January 1993, through September 1995, have been linked epidemiologically to improperly cooked ground beef (CDC, 1996). Ground beef accounts for nearly 50% of the beef consumed in the US (Marriott et al., 1980).

Recently, there has been substantial research pertaining to the heat resistance of *E. coli* O157:H7 in meat and the reported D-values at 50–64°C range from 92.67 to 0.16 min (Ahmed et al., 1995; Line et al., 1991; Doyle and Schoeni, 1984). These studies clearly indicate that the organism does not have an unusual heat resistance.

Destruction of microorganisms by heat has traditionally been described as log-linear in nature, i.e. at a given temperature the logarithm of bacterial numbers decline linearly with time (Stumbo, 1973; Tomlins and Ordal, 1976). However, deviations from the linear declines in the log numbers with time have been frequently observed (Pflug and Holcomb, 1983; Tomlins and Ordal, 1976). These reports clearly indicate that survival curves exhibit an initial lag period or shoulder, i.e. time periods where the bacterial population remain at the inoculation level, followed by exponential decline. In some instances a tailing or a subpopulation of more persistent bacteria is observed that decline at a slower rate than the majority of the cells. The data cannot be accounted for by experimental artifacts and there is presently no satisfactory, unifying explanation for the variability in thermal death kinetics. Typically, a linear regression approach is employed to analyze such thermal inactivation data despite a poor fit. This certainly results in false estimates of heat resistance values for non-linear survival curves. For non-linear survival curves, a model was derived by Whiting (1993) from the logistic-based model of Kamau et al. (1990) to include a shoulder and two populations (a major population and a subpopulation). This approach is an ideal way for estimating the heat resistance for non-linear heat inactivation data.

In the work reported here, we determined D-values in 90% lean beef and chicken by using (a) linear regression from the straight line portion of the survival curves, and (b) by a survival equation/model that was fitted to the non-linear survival curve to obtain two D-values, one for the major population and another for a subpopulation. We also assessed the change in heat resistance of *E. coli* O157:H7 in beef during refrigerated and frozen storage.

2. Materials and methods

2.1. Organisms

The four strains of *E. coli* O157:H7 used in this study: EDL-931, 45753-35, C1-9218 and 933, were obtained from the Microbial Food Safety Research Unit laboratory culture collection. Individual stock cultures were maintained on brain heart infusion agar (BHI; Difco, Detroit, MI) slants at 4°C with monthly transfers to maintain their viability.

2.2. Ground meat

Raw 90% lean ground beef and lean ground chicken were obtained from a local supermarket and frozen (-5°C) until use (≈ 60 days). Prior to inoculation with the four-strain mixture of *E. coli* O157:H7, the meats were thawed at 4°C for 24 h.

2.3. Inoculum preparation

Each strain was cultured individually in 50 ml BHI broth in 250 ml flasks incubated aerobically at 37°C. Two consecutive 24 h transfers were made using 0.1 ml inocula. Final late stationary phase cultures were centrifuged ($5000 \times g$, 15 min, 4°C) and washed in 0.1% peptone water (w/v); this centrifugation and washing was repeated two

times. The cell pellets were finally suspended in peptone water to a target level of 9 $\log_{10}$ cfu/ml. The population densities in each inoculum suspension was enumerated by spiral plating (Spiral Biotech, Bethesda, MD; Model D) appropriate dilutions in 0.1% peptone water (in duplicate) on tryptic soy agar (TSA; Difco) plates to determine the initial number of bacteria. Equal volumes of each culture were combined in a sterile test tube to obtain a four strain mixture of *E. coli* O157:H7 (9 $\log_{10}$ cfu/ml) prior to inoculation of meat.

2.4. Sample preparation, inoculation, and storage

Duplicate 3-g ground beef or chicken samples were aseptically weighed into 15 × 22.9 cm sterile Whirl-pak™ sampling bags (Model B736; NASCO, Modesto, CA) and inoculated with 0.1 ml of an appropriate dilution of *E. coli* O157:H7 cocktail so that the final concentration of cells was approximately 7 $\log_{10}$ cfu/g. Negative controls included bags containing meat samples inoculated with 0.1 ml of 0.1% (w/v) peptone water with no bacterial cells. Thereafter, the bags were manually mixed to ensure even distribution of the organisms in the meat sample, compressed into a thin layer ($\approx$ 1–2 mm thick) by pressing against a flat surface, excluding most of the air, and then heat-sealed. To determine the influence of storage temperatures on the heat resistance of *E. coli* O157:H7, ground beef samples were frozen (− 18°C) and refrigerated (4°C) for 48 h. Bags containing meat samples that were not refrigerated or frozen were designated as fresh meat samples. Frozen and refrigerated meat samples were thawed/equilibrated to room temperature prior to heating to an internal temperature of 60°C.

2.5. Thermal inactivation

Bags at room temperature were placed in a basket and then fully submerged in a temperature-controlled water bath (Exacal, Model Ex-251HT, Neslab Instruments, Newington, NH) stabilized at 55, 57.5, 60, 62.5 or 65°C. The temperature was continuously monitored by two copper-constantan thermocouples inserted, prior to heat sealing, at the center of two uninoculated bags. The thermocouple readings were measured and recorded using a Keithyl-Metrabyte data logger Model DDL 4100 (Tauton, MA) connected to a microcomputer. The thermocouple signal was sampled every second, and the two readings were averaged to determine the bag internal temperature. Come-up times, which were negligible, were included as part of the total heating time when these were used to calculate the D-values. Two bags for each replicate were then removed at designated time intervals with the sampling frequency being based on the heating temperature, e.g. 10 min at 55°C; 0.5 min at 65°C. Total heating time ranged from 120 min at 55°C to 4 min at 65°C. After removal, bags were immediately plunged into an ice-water bath and analyzed within 30 min.

2.6. Enumeration of surviving bacteria

For determination of the number of surviving bacteria, sterile 0.1% peptone (3 ml) was combined with each meat sample to obtain a 1:1 (w/v) slurry and pummelled for 1 min with a Stomacher 400 Lab-blender (Tekmar, Cincinnati, OH). Decimal serial dilutions were prepared in 0.1% peptone water and appropriate dilutions were surface plated onto agar dishes containing TSA using a spiral plater (Model D; Spiral Biotech, Bethesda, MD). Samples not inoculated with *E. coli* O157:H7 were plated as controls. Also, 0.1 ml of undiluted suspension was surface plated, where relevant. After 120 min resuscitation at room temperature to allow for the recovery of heat-damaged cells, the TSA plates were overlaid with 10 ml of Sorbitol MacConkey agar (SMA, Oxoid) pre-tempered to 47°C. After overlaying, the plates were allowed to solidify before incubating at 35°C. Typical *E. coli* O157:H7 colonies were counted after 48 h of incubation. Isolates from plates were randomly selected and subjected to serological confirmation as *E. coli* O157:H7 serotype (RIM, *E. coli* O157:H7 Latex Test; Remel, Lenexa, KS). For each replicate experiment performed in duplicate, an average cfu/g of four platings of each sampling point was used to determine the D-values.

D-values (time to inactivate 90% of the population) were calculated from the straight portion of the survival curves by plotting log of survival counts vs their corresponding heating times using Lotus 1-2-3 Software (Lotus Development, Cambridge, MA). Only survival curves with more than five values in the straight portion, with a correlation coefficient (r^2) > 0.90, and descending more than 5 log cycles were used. Also, regression lines were fitted to experimental data points that contributed to tailing or shouldering by a survival equation (model) developed by Whiting (1993) using Gauss-Newton curve fitting program (Abacus Software Program, ERRC, USDA, Philadelphia, PA) and two D-values were calculated. The z-values were estimated by computing the linear regression (Ostle and Mensing, 1975) of mean $\log_{10}$ D-values vs their corresponding heating temperatures using Lotus 1-2-3 software.

2.8. Statistical analysis

The heat resistance data were analyzed by analysis of variance (ANOVA) using SAS (SAS Institute, 1989) to determine if there were statistically significant differences among the treatments. Bonferroni mean separation test was used to determine significant differences ($P < 0.05$) among means (Miller, 1981).

3. Results and discussion

The pH of the 90% lean ground beef and lean ground chicken used in the study was around 6.0. Surviving *E. coli* O157:H7 cells/g of beef or chicken were determined and logarithms were plotted against exposure time at the test temperature. A representative example of the survivor curve exhibited in Fig. 1 demonstrates a linear decrease in population at 60°C when the heating menstruum was fresh chicken. In contrast, when *E. coli* O157:H7 was heated at 60°C in beef, inactivation kinetics showed deviations from the log-linear decline in surviving cells with time.

While inactivation of microorganisms by heat has traditionally been described as log-linear in nature, researchers have consistently reported that there are significant deviations from expected kinetics of thermal death (Hansen and Riemann, 1963). Attempts have been made to explain these deviations by various theories and models (Ott et al., 1961; Casolari, 1988; Gould, 1989; Pflug, 1990) and has been excellently reviewed by Whiting (1995). Hansen and Riemann (1963) suggested that the deviations in linear survival curves result from a cell population heterogenous in heat resistance.

The D-values of *E. coli* O157:H7 in beef and chicken at 55, 57.5, 60, 62.5 and 65°C were determined. The D-values, obtained by linear regression, in beef ranged from 21.13 min at 55°C to 0.39 min at 65°C (Table 1). Regression curves calculated for the five temperatures (55, 57.5, 60, 62.5 and 65°C) fit with an r^2 value of > 0.90. Using a survival model, D-values in beef ranged from 20.45 min (D_1 and there was no D_2) at 55°C to 0.16 min (D_1) and 1.45 min (D_2) at 65°C (Table 1). When *E. coli* O157:H7 was heated in chicken, D-values calculated by both approaches were significantly less ($P < 0.05$) at all temperatures (Table 2). Beef offered protection compared to chicken, indicated by higher recovery of heated *E. coli* O157:H7 cells in case of beef. The increased thermal resistance of *E. coli* O157:H7 in beef compared to chicken may be attributed to the effect of different species and the differences in fat

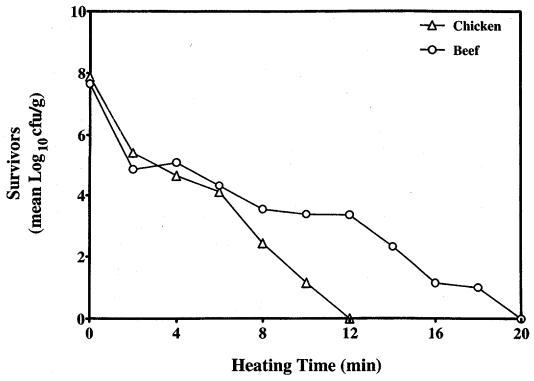

Fig. 1. Survivor curve of the four strain cocktail of *E. coli* O157:H7 heated in beef and chicken at 60°C.

Table 1
Heat resistance (expressed as D-values in min) for *Escherichia coli* 0157:H7 4-strain mixture in ground beef at 55–65°C

Temp (°C)	Method to determine D-value[a]			
	Linear regression	Curve fitting		
	D-value (r^2)[b]	D_1[c]	D_2[d]	RMS error[e]
55	21.13 ± 0.25 (0.99)	20.45 ± 0.26	—[f]	0.20
57.5	4.95 ± 0.16 (0.93)	2.70 ± 0.82	7.83 ± 0.90	0.29
60	3.17 ± 0.18 (0.94)	0.61 ± 0.82	4.54 ± 1.07	0.18
62.5	0.93 ± 0.01 (0.93)	0.39 ± 0.01	1.37 ± 0.03	0.18
65	0.39 ± 0.00 (0.91)	0.16 ± 0.01	1.45 ± 0.58	0.25

[a] D-values shown are the means of two replicate experiments, each performed in duplicate and expressed as mean ± standard deviation.
[b] Correlation coefficients in parenthesis.
[c] D-value of a major population.
[d] D-value of subpopulation.
[e] Root mean squares error.
[f] Curve was linear.

content between the substrates. The z-values in beef and chicken ranged from 4.94–6.79°C, with the exception of the z-value (9.25°C) of the subpopulation in beef (see Fig. 2). Since *E. coli* O157:H7 exhibited no tailing at 55–60°C in chicken, the z-values of the subpopulation could not be calculated.

By selecting a common test temperature, it is feasible to compare data published in the literature on the heat resistance of *E. coli* O157:H7. While we used a survival equation for non-linear survival curves to obtain D-values of the tailing region ($D_{60°C} = 4.54$ min) in addition to the D-values of the major population ($D_{60°C} = 0.61$ min), the previous studies (Doyle and Schoeni, 1984; Ahmed et al., 1995) calculated D-values using only linear regression analysis for the best fit line of the survivor curve. Doyle and Schoeni (1984) reported D-values at 60°C of 0.75 min for *E. coli* O157:H7 strain 932 inoculated into ground beef containing 17–20% fat to give a final concentration of 7 $\log_{10}$ cfu/g. In these experiments, the meat was heated in pyrex test tubes capped with rubber stoppers. Ahmed et al. (1995) reported that the D-value of *E. coli* O157:H7 in ground beef heated at 60°C in thermal death time tubes ranged from 0.45 (beef, 7% fat) to 0.47 (beef, 20% fat) min; the values ranged from 0.38 (chicken, 3% fat) to 0.55 (chicken, 11% fat) in chicken. Slight differences in D-values obtained in our study and those reported by previous workers may be attributed to different *E. coli* O157:H7 strains (assessed individually or as a mixture), physiological condition of the cells, fat content of meat, and methodology used for the detection of survivors.

To assess the influence of storage conditions on the heat resistance of *E. coli* 0157:H7, ground beef containing *E. coli* O157:H7 was frozen or refrigerated for 48 h before exposure to heat to an internal temperature of 60°C. Fig. 3 depicts the destruction of *E. coli* O157:H7 expressed as log of the ratio of count at time t (N) and initial count (N_0). This was calculated by subtracting the log initial count before cooking ($\log N_0$) from the log final count after heating ($\log N$). The resulting data gave the log numbers of *E. coli* O157:H7 colonies per gram of beef destroyed by the heat treatment. For fresh (not refrigerated or frozen) ground beef heated at 60°C, cells of *E. coli* O157:H7 decreased by 2.48 logs (7.69 $\log_{10}$ cfu/g to 5.21 $\log_{10}$ cfu/g) within 2 min and by 6.7 logs (< 1 $\log_{10}$ cfu/g) at 16 min (Fig. 3). In comparison to the fresh ground beef, heating refrigerated and frozen ground beef at 60°C for 2 min resulted in a 0.57 and 0.21 log reductions in *E. coli* O157:H7 colony counts/g from an initial inocu-

Table 2
Heat resistance (expressed as D-values in min) for *Escherichia coli* 0157:H7 4-strain mixture in ground chicken at 55–65°C

Temp (°C)	Method to determine D-value[a]			
	Linear regression	Curve fitting		
	D-value (r^2)[b]	D_1^c	D_2^d	RMS error[e]
55	11.83 ± 0.10 (0.99)	11.56 ± 0.08	—[f]	0.37
57.5	3.79 ± 0.05 (0.98)	3.26 ± 0.03	—	0.23
60	1.63 ± 0 (0.99)	1.59 ± 0	—	0.45
62.5	0.82 ± 0.01 (0.95)	0.48 ± 0.07	1.31 ± 0.08	0.32
65	0.36 ± 0 (0.93)	0.21 ± 0.01	0.52 ± 0.22	0.29

[a] D-values shown are the means of two replicate experiments, each performed in duplicate and expressed as mean ± standard deviation.
[b] Correlation coefficients in parenthesis.
[c] D-value of a major population.
[d] D-value of subpopulation.
[e] Root mean squares error.
[f] Curve was linear.

lum of 7.85 and 7.56 $\log_{10}$ cfu/g, respectively; the log destruction was 5.65 and 5.05 $\log_{10}$ cfu/g at 16 min. The slopes of the inactivation curves between 2 and 16 min of heating were very similar for fresh, refrigerated and frozen samples (Fig. 3). This observation with refrigerated and frozen ground beef is not in agreement with those made by other researchers (Jackson et al., 1996). These authors reported that the cells in frozen (−18°C) patties were more resistant to heat ($P < 0.05$) than those under refrigeration (3°C) storage. These investigators stored inoculated patties (114 g) at −18°C for 8 days and at 3°C for 9 h; in contrast, we stored inoculated ground beef (3 g) at −18°C and 4°C for 48 h. The inconsistent results between the former study by Jackson et al. (1996) and our study may also be attributed to sample size differences 114 vs 3 g, resulting in substantially different rates of heating during cooking, or to the cold-shock protein induction and biochemical changes in the bacterial membranes as a result of storage at low temperatures for different time periods.

The data presented in Tables 1 and 2 can be used to predict the time required at specified temperatures to achieve a certain number of log-cycle reductions of *E. coli* O157:H7 when heated in 90% lean beef, or lean chicken. Based on the thermal death time values determined in this study, contaminated 90% lean beef should be heated to an internal temperature of 65°C for at least 7.25 min and lean chicken for 2.6 min; this is

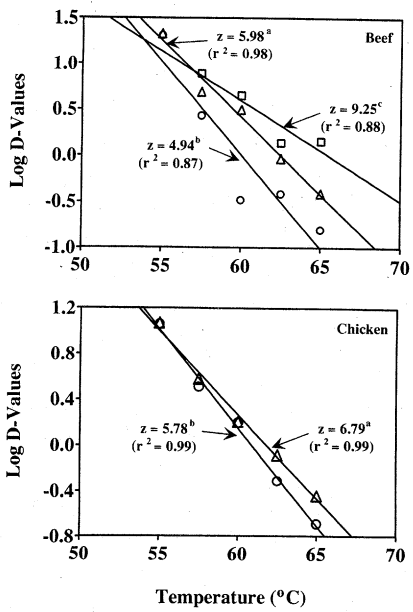

Fig. 2. Thermal-death-time curves (z-values) for *E. coli* O157:H7 over the temperature range 55–65°C. The D-values, [a] calculated by linear regression, [b,c] D_1 (D-value of a major population) and D_2 (D-value of subpopulation), calculated by curve fitting in beef and chicken, used to determine the z-values were the means of two replicates and were obtained based on survivors on the recovery medium.

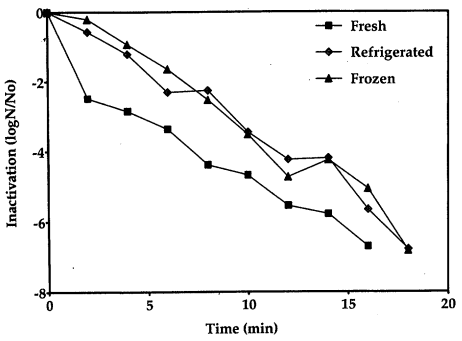

Fig. 3. Destruction of *E. coli* O157:H7 in fresh, refrigerated and frozen ground beef at 60°C. The data has been expressed as log of the ratio of count at time t (N) and initial count (N_0).

based on the argument that thermal treatments must be designed to achieve a 5-D process for *E. coli* O157:H7. Thermal death time values from this study will assist food processors in designing acceptance limits on critical control points that ensure safety against *E. coli* O157:H7 in cooked beef and chicken.

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